Effect of rhDNase on Airflow Obstruction and Mucociliary Clearance in Cystic Fibrosis

BETH L. LAUBE, ROBERT M. AUCI, DOUGLAS E. SHIELDS, DAVID H. CHRISTIANSEN, MELISSA K. LUCAS, HENRY J. FUCHS, and BERYL J. ROSENSTEIN

Departments of Environmental Health Sciences and Pediatrics, The Johns Hopkins Medical Institutions, Baltimore, Maryland; and Genentech Inc., South San Francisco, California

We tested the hypothesis that recombinant human deoxyribonuclease 1 (rhDNase) reduces airflow obstruction and improves mucociliary clearance in patients with cystic fibrosis (CF), and that improvements seen in FEV_1 and FVC after rhDNase treatment are independent of chest physical therapy (CPT). CF patients inhaled placebo (10 patients) or 2.5 mg rhDNase aerosol (10 patients) twice a day for six consecutive days. Compared with baseline, there were no statistically significant differences between the two study groups by Day 6 for indices of airflow obstruction obtained from gamma-camera images of the right lung following inhalation of ^{99m}Tc aerosol, or for mucociliary clearance or the rate of clearance of the radioaerosol, quantified over a 6-h period. By Day 6, FEV_1 and FVC were significantly higher in the rhDNase-treated group than in the placebo group, increasing by an average of 9.4 ± 3.5% and 12.7 ± 2.6%, respectively, as compared with a decrease of 1.8 ± 1.7% and an increase of 0.4 ± 1.1%, respectively ($p < 0.05$). There was no significant change in the FEV_1/FVC ratio on Day 6 (0.68 ± 0.05) compared with baseline (0.70 ± 0.05) in the rhDNase group. On Day 6, FEV_1 and FVC decreased after CPT in both study groups, but the decreases were not significant. Our results indicate that aerosolized rhDNase improves FEV_1 and FVC independent of CPT. We were unable to demonstrate that rhDNase reduces airflow obstruction or improves mucociliary clearance. **Laube BL, Auci RM, Shields DE, Christiansen DH, Lucas MK, Fuchs HJ, Rosenstein BJ. Effect of rhDNase on airflow obstruction and mucociliary clearance in cystic fibrosis.**

AM J RESPIR CRIT CARE MED 1996;153:752–60.

A defect in the cystic fibrosis (CF) gene on chromosome 7, which regulates electrolyte transport in lung epithelial cells, leads to a threefold increase in transepithelial sodium absorption and a decrease in chloride secretion in CF patients (1). This abnormality is associated with the production of thick, viscous mucus that favors bacterial colonization, the accumulation of inflammatory cells, airway damage, and airways obstruction. Deoxyribonucleic acid (DNA), released by inflammatory cells at concentrations of 3 to 12 mg/ml (2, 3), further increases the viscosity of CF sputum. *In vitro*, the addition of purified recombinant human DNase 1 (rhDNase) to CF sputum hydrolyzes extracellular DNA, reduces the viscoelastic properties of the sputum, and transforms the sputum within minutes from a nonflowing viscous gel to a flowing liquid (4). Results of a Phase II, multicenter, placebo-controlled, double-blind, randomized study showed that 10-d administration of three doses of aerosolized rhDNase (0.6, 2.5, or 10.0 mg twice daily) improved FVC by 10 to 12% and FEV_1 by 10 to 15% in a group of 181 CF patients (5). A Phase III multicenter study demonstrated that administration of 2.5 mg rhDNase once or twice daily reduced the risk of respiratory tract exacerbations requiring parenteral antibiotics by 28% and 37%, respectively, relative to placebo over a 24-wk period in 968 patients with CF (6). In addition, FEV_1 increased by approximately 6% in rhDNase-treated patients. Taken together, these data indicate that the health status of some patients with CF appears to be significantly improved by the administration of rhDNase. Nevertheless, the mechanism by which rhDNase improves pulmonary function and reduces the rate of respiratory exacerbations is unclear.

Studies using gamma-camera imaging technology have shown that CF patients and other patients with airflow obstruction deposit radiolabeled aerosols inhomogeneously as compared with normal subjects (7–12). In a previous study, we demonstrated that inhomogeneity in aerosol deposition, quantified by changes in the deposition parameter known as skew, was significantly reduced in a group of CF patients with severe airways obstruction following hospitalization for a pulmonary exacerbation and intensive treatment with antibiotics, bronchodilators, and chest physical therapy (CPT) (13). The FEV_1/FVC ratio (an index of airflow obstruction) was also improved. These results suggested that improved homogeneity in aerosol deposition may have been related to a reduction in airflow obstruction, perhaps because of decreased mucoid impaction or atelectasis.

In the present study, we hypothesized that one mechanism by which rhDNase treatment improves pulmonary function is by altering CF mucus such that regional and/or local obstruction to airflow within the lungs is reduced. We tested this hypothesis by quantifying the distribution homogeneity of a radiolabeled aerosol in the lungs of rhDNase-treated patients and those given

(*Received in original form March 20, 1995 and in revised form September 1, 1995*)

Supported by Genentech Inc., South San Francisco, California.

Correspondence and requests for reprints should be addressed to Beth L. Laube, Ph.D., The Johns Hopkins University, School of Public Health, 615 N. Wolfe Street, Room 2001, Baltimore, MD 21205.

placebo, using gamma-camera imaging techniques. Alternatively, we hypothesized that rhDNase treatment improves pulmonary function in CF by improving mucociliary clearance mechanisms. We tested this hypothesis by quantifying the removal of the radiolabeled aerosol from the lungs of the two study groups over time. It was assumed that measurement of changes in mucociliary clearance would be valid only if deposition indices did not change as a result of treatment. Thus, if the deposition pattern were significantly altered as the result of rhDNase treatment (i.e., a central deposition pattern became more of a peripheral pattern), it would not be possible to distinguish between the effect of rhDNase and deposition location on mucociliary clearance rate. This is because deposition pattern *per se* is a major determinant of the mucociliary clearance of material deposited in the lungs, and changes in deposition pattern cannot be minimized by a normalization procedure.

We also hypothesized that improvements seen in FEV_1 and FVC after rhDNase treatment are independent of mechanical clearance mechanisms resulting from CPT. We tested this hypothesis by comparing FEV_1 and FVC measurements before and after CPT in the treatment and placebo groups.

METHODS

Protocol

The protocol described here was approved by the Joint Committee on Clinical Investigations of the Johns Hopkins Medical Institutions. Informed consent was obtained from each volunteer. The study was a single-site, double-blind, placebo-controlled trial in which patients were randomized to receive 2.5 mg of rhDNase aerosol or placebo aerosol twice a day for six consecutive days. The rhDNase and placebo aerosols were generated from 2.5 ml of rhDNase excipient (placebo) or 2.5 mg of rhDNase in a 2.5 ml solution, using a Hudson T Up-Draft II nebulizer (Hudson RCI, Irvine, CA) that was attached to a Pulmo-Aid air compressor (DeVilbiss, Somerset, PA). During a 10- to 15-min treatment period, aerosol was generated continuously by the compressor. Patients inhaled either placebo or rhDNase on a 6:00 to 10:00 A.M. and 6:00 to 10:00 P.M. schedule. Each patient was instructed to inhale drug at the same time each day. Morning doses on Days 1, 2, and 6 were given at the study center. The remaining doses were self-administered at home.

The effect of CPT on changes in pulmonary function was assessed through spirometric measurements at baseline (3 to 5 d prior to the first day of treatment) and before and after CPT on Days 2 and 6. The study drug was inhaled before CPT. At least 1 h separated CPT from the second spirometric measurement.

The effect of rhDNase on aerosol distribution homogeneity and mucociliary clearance was quantified from gamma-camera images of the lungs at baseline and on Days 2 and 6. Radioaerosol inhalation occurred after the second spirometric measurement. Sequential images of the lungs were obtained immediately after radioaerosol inhalation and for 6 h thereafter.

Patient Population

All patients were nonsmokers and had a documented sweat chloride concentration > 60 mEq/L by quantitative pilocarpine ionophoresis and one finding consistent with the clinical diagnosis of CF. Other inclusionary criteria were either male or female sex, age > 18 yr, an FVC between 35 and 75% predicted for height and age or a Brasfield chest radiograph score (14) of < 20 within 7 d of receiving drug, an ability to perform and reproduce two FVC and FEV_1 maneuvers, each within 0.15 L of each other, performance of daily CPT at the time of the study, and a stable antibiotic, bronchodilator, and/or corticosteroid regimen within 14 d before receiving the study drug.

Efficacy Assessments

Aerosol distribution homogeneity. The effect of rhDNase on aerosol distribution homogeneity was quantified from a large, field-of-view gamma-camera (Technicare Omega 500; Solon, OH) image of the lungs following the inhalation of a radioaerosol at baseline and on Days 2 and 6. The radioaerosol was generated by the Hudson nebulizer from a 0.9% saline solution containing 10 to 15 mCi of ^{99m}Tc sulfur colloid (Syncor, Inc., Baltimore, MD). ^{99m}Tc sulfur colloid was chosen as the radiolabeled preparation for the aerosol experiments because it is a non-diffusable agent that either remains in the lungs and whose radioactivity decays to zero activity over 1 to 2 d, or is cleared from the lungs via the mucociliary pathway over several hours, swallowed, and subsequently excreted from the gastrointestinal tract.

The Hudson nebulizer was attached to a Pulmo-Aid air compressor via a nebulization dosimeter (Rosenthal-French, Baltimore, MD) that regulated the time of each air pulse from the compressor. Using the dosimeter, the nebulizing airflow used for aerosolization was activated manually for 1.8 s. Patients inhaled the radioactive bolus while inspiring dilution air from the bell of a Stead-Wells spirometer (Warren E. Collins, Braintree, MA) at 0.5 L/s, beginning at 50% vital capacity. Inspiration continued until patients attained their total lung capacity. This method of aerosol inhalation has been described in detail previously (15). At the end of each inhalation, patients removed their mouths from the nebulizer and exhaled normally. They were instructed not to hold their breath. Patients repeated this inhalation procedure several times to achieve adequate radiolabeling of the lungs. In order to reduce the possibility of radiolabeling of the esophagus prior to the completion of the counting process, each patient rinsed his or her mouth with water and expectorated the rinse before the counting process began. The patients also ate a small pastry and drank water prior to the commencement of the counting process, in order to wash any radioactivity remaining in the mouth and esophagus into the stomach.

Aerosol particle size determination. Because it was not possible to radiolabel the rhDNase itself, the radioaerosol served as a surrogate for the rhDNase aerosol. It was therefore assumed that the deposition pattern of the radioaerosol reflected that of the rhDNase aerosol. For this assumption to be accurate, the particle-size distribution of the two aerosols had to be similar. In the case of rhDNase, aerosol was generated continuously as described earlier, whereas the radioaerosol was generated only at the time of inspiration by means of the dosimeter. By controlling the time of each aerosolization with the dosimeter, it was possible to control the dose of radioactivity delivered per actuation. However, the use of the dosimeter could modify the particle-size distribution produced by the nebulizer. For this reason, a Malvern Master Sizer (Malvern, Inc., Amherst, MA) was used to measure the particle-size distribution of aerosol generated with either continuous or intermittent flow. The total number of sweeps of the Malvern detector elements was adjusted so as to match the time frame of the aerosol pulse delivered by the dosimeter. The median diameters of the particles produced from 1 mg/ml rhDNase solution and ^{99m}Tc sulfur colloid for both continuous and intermittent delivery were compared.

Gamma-camera imaging procedures. Patients discontinued inhaled bronchodilators 12 h and oral bronchodilators 24 h before each imaging procedure. At baseline, patients underwent the following gamma-camera imaging procedures of the posterior lungs: (*1*) a 5-min transmission scan to provide lung boundaries; (*2*) a 300,000-count ventilation scan while rebreathing xenon-133 gas generated by a Pulmonex Xenon System (Atomic Products, Corp., Shirley, NY), to provide a lung volume calibration; and (*3*) a 5-min aerosol scan after the inhalation of ^{99m}Tc sulfur colloid to provide images for the analyses of distribution homogeneity and mucociliary clearance. On Days 2 and 6 of treatment, the aerosol imaging procedure was repeated. Lung images were acquired in a 256 × 256 picture element (pixel) matrix. Images were stored on a General Electric Star II computer (St. Albans, Hertfordshire, England) for processing.

Determinations of aerosol distribution homogeneity. Images of the right lung were analyzed in terms of: (*1*) values of skew (a measure of distribution asymmetry); (*2*) the ratio of counts of radioactivity detected in an inner zone (composed anatomically of large and small airways and alveoli) and an outer zone (small airways and alveoli) (I:O ratio); and (*3*) the ratio of counts of radioactivity detected in an apical and basal third of the lung (A:B ratio). Lower skew values indicated enhanced deposition homogeneity on a per pixel, or local basis, within the lung. I:O and A:B ratios closer to 1.00 indicated increased homogeneity in aerosol deposition on a more regional basis. Analyses of the left lung were considered unreliable because radiolabel that was swallowed appeared in the gastrointestinal tract, and this activity often overlapped with activity in the left lung image.

Skew Analysis

Frequency-distribution histograms were constructed from deposition images of the right lung at baseline and on Days 2 and 6, as previously described (13). The number of pixels with a given count value (expressed as a percentage of total pixels) appeared on the y axis and the count values appeared on the x axis. Histograms were analyzed for skew using an equation described previously (13). Change in skew for each patient was defined in absolute terms as:

$$\text{Change in skew} = \text{Skew (time X)} - \text{Skew (baseline day)}. \quad (1)$$

Regional Analyses

The right lung border and inner, outer, apical, and basal zone boundaries were first delineated in the lung transmission scan. These regional borders were then superimposed onto the ventilation scan and the two aerosol scans. The transmission scan was used to define the lung borders because the lung boundaries shown in the ventilation scan in some patients with severe airway obstruction were incomplete. By using the transmission scan, it was possible to define the lung boundaries on the subsequent three scans, making regional data in each of the scans comparable.

Using a method similar to one described by Dolovich and colleagues (8) and Chung and associates (9), the right lung in the transmission scan was divided into three segments, yielding inner and outer zone regions. Previously, we used a similar method of analysis to quantify the regional distribution of radioaerosol in normal subjects following exposure to acid fog (16). To obtain the apical and basal zones, the height of the right lung was divided into three equal segments, as described previously by Agnew and associates (10). Mean counts per pixel in each of the four regions were calculated, and the I:O and A:B ratios for both the ventilation and aerosol scans were derived. The aerosol scan ratios were divided by the ventilation scan ratios to correct for differences in lung volume within the various regions. Changes from baseline for the I:O and A:B ratio for each patient were defined in absolute terms as described earlier for skew.

Mucociliary Clearance

The effect of rhDNase on mucociliary clearance was quantified by sequential imaging of the right lung over a 6-h period. Measurements of mucociliary clearance during this time frame reflected removal of the radiolabel from large and small ciliated airways, since alveolar clearance occurs over a period of days. The clearance of the tag from the right lung was expressed in terms of its reciprocal (retention). The greater the retention of the radiolabel, the less was cleared from the lung by mucociliary clearance mechanisms. Retention was quantified as the percentage of the initially deposited radioactivity remaining in the lung at 15, 30, and 45 min, and after 1, 2, 4, and 6 h. Percent retention was computed for the total right lung and for the inner zone of the right lung as follows:

$$\%\ \text{Retention at time X} = 100\%\ \text{at time 0} - \%\ \text{cleared at time X}. \quad (2)$$

In addition, clearance rates (retention slopes) for the total right lung and for the inner zone of the right lung were computed and compared for each treatment group for the intervals 0 to 1 h, 1 to 2 h, and 2 to 6 h.

Coughing during the 6-h measurement period was a potential confounder of the quantification of the retention of the radioaerosol, since mechanical clearance of the radiolabel by coughing could not be differentiated from clearance by mucociliary movement. For this reason, all patients were given "cough cards" at the beginning of each study. Patients were instructed to record every cough that occurred throughout the 6 h after radioaerosol administration by making a checkmark on the card. The number of checkmarks was tallied for each study day for each patient.

Pulmonary Function

Measurements of FEV_1 and FVC were assessed to determine the effect of rhDNase on FEV_1 and FVC and the effect of CPT on FEV_1 and FVC. The study drug was inhaled after the first set of spirograms and before CPT. At least 1 h separated CPT from the second spirometric measurement. Pulmonary function testing was performed according to the guidelines of the American Thoracic Society (ATS) (17). The mean of the pulmonary function measurements on the two screening visits and on Day 1 of treatment (pre-CPT) was used as the baseline value in order to minimize intrapatient variability. FEV_1 and FVC were measured in liters and converted to percent-predicted values based on height, age, and sex-specific population standards as developed by Knudson and colleagues (18). The study center was instructed to use the same testing equipment for each patient throughout the study and, preferably, the same testing personnel at every visit. The percent change in FEV_1 from baseline to each pre-CPT treatment measurement was defined as follows:

$$\%\ \text{Change in FEV}_1\ \text{at time X} = \frac{(\text{FEV}_1\ \text{at time X} - \text{baseline FEV}_1)}{\text{baseline FEV}_1} \times 100 \quad (3)$$

The same calculation was performed for percent change in FVC.

Chest Physiotherapy

Chest physiotherapy was performed at the study center by a registered physical therapist. All patients received the same type of CPT at the study site, regardless of the type of CPT that was performed at home. When not required to be at the study site, patients continued their individual CPT routine at home. The effect of CPT on the FEV_1 and FVC measurements was quantified as the difference in percent change from baseline between the pre- and post-CPT values at Days 2 and 6 (post-CPT minus pre-CPT).

Statistical Analyses

Initial comparability of the study groups with regard to baseline demographic and disease characteristics was assessed with the *t* test (19) for continuous variables and with chi-square tests for categorical data (19). Study groups were compared with respect to changes in skew and I:O and A:B ratios from baseline to Days 2 and 6, using the *t* test (19). Changes in retention of the radiolabel in the total right lung as well as in the inner zone were calculated for each time point and compared using ANOVA methods. The *t* test was used to compare: (*1*) changes between study groups in the clearance rate from baseline to Days 2 and 6 for each of the time periods; (*2*) changes between study groups in the number of coughs from baseline to Days 2 and 6; (*3*) the percent change between study groups in FEV_1 and FVC from baseline to the pre-CPT treatment measurement on Days 2 and 6; and (*4*) percent changes between study groups in FEV_1 and FVC (post-CPT minus pre-CPT) from baseline to Days 2 and 6. Data are presented as means ± SE; differences between means were considered significant at $p < 0.05$.

RESULTS

Our results are presented as a comparison between baseline data and data obtained on Day 6 of treatment. With the exception of CPT effects, results from data obtained on Day 2 of treatment were similar to those obtained on Day 6, and, for that reason, are not presented here.

Study Population

Twenty-one patients were screened for entry into the study; one patient was found to be ineligible. A total of 20 patients were randomized, of whom 10 were assigned to placebo and 10 were assigned to rhDNase. All 20 patients completed the 6-d study.

Demographics and Baseline Characteristics

The demographics and baseline characteristics of the two study groups were similar (Table 1). Patients randomized to the rhDNase treatment group had slightly lower mean baseline FEV_1 and FVC values, NIH clinical scores (20), Brasfield chest radiograph scores (14), and O_2 saturation than did those in the placebo group, but none of the values were significantly different between the two groups. There was no clinically significant difference in medication use or physical therapy routine between the two groups.

Particle Size Determinations

The volume median diameter for rhDNase aerosol generated via the Pulmo-Aid air compressor averaged 4.47 μm, which was not

TABLE 1

BASELINE CHARACTERISTICS

	Placebo (*n* = 10)	rhDNase 2.5 mg BID (*n* = 10)
Mean age, yrs	27.8	24.2
Range	18–44	18–32
% Male	30	60
% Female	70	40
Mean Height, cm	167.2	170.5
Mean body mass index, kg/m^2		
Males	21.6	19.4
Females	20.7	20.2
Mean FEV_1, % predicted	58.8	50.9
Mean FVC, % predicted	76.9	70.9
Mean NIH score	79.1	75.4
Mean Brasfield score	16.8	15.6
Mean O_2 saturation	98.2	97.5
Ratios		
Inner:outer - right lung	1.59	1.53
Apex:basal - right lung	0.80	0.75
Skew - right lung	0.68	0.79
Retention		
Hr 1- right lung	90.0	90.0
Hr 6 - right lung	82.0	86.1

different from the average diameter generated by the dosimeter–Pulmo-Aid combination (3.78 μm).

Changes in Aerosol Distribution Homogeneity

Mean values for skew, I:O, and A:B ratios at baseline and Day 6 are shown in absolute terms in Figures 1 through 3, respectively, for the placebo group and for rhDNase-treated patients. At baseline, skew averaged 0.68 ± 0.09 and 0.79 ± 0.12 for the placebo group and the rhDNase-treated group, respectively. By Day 6, the average skew value was 0.82 ± 0.14 in the placebo group and 0.78 ± 0.09 in the rhDNase-treated patients.

At baseline, the I:O ratio averaged 1.59± 0.14 and 1.53 ± 0.20 for the placebo group and for rhDNase-treated patients, respectively. By Day 6, the average was 1.64 ± 0.17 in the placebo group and 1.51 ± 0.14 in the rhDNase-treated patients.

At baseline, the A:B ratio averaged 0.80 ± 0.15 and 0.75 ± 0.20 for the placebo group and for rhDNase-treated patients, respectively. By Day 6, the average was 0.86 ± 0.10 in the placebo group and 0.83 ± 0.28 in the rhDNase-treated patients. There were no statistically significant differences between the treatment groups for skew, I:O ratio, or A:B ratio.

Changes in Mucociliary Clearance

Figure 4 shows the mean percent retention of the radiolabel in the total right lung for each time point in the two study groups at baseline and on Day 6. The percent retention at all time points was similar and unchanged for the two study groups.

There were no significant differences in the clearance rate of the radiolabel, measured in terms of the slope of retention for the total right lung between 0 and 1 h, and 1 to 2 h, and 2 and 6 h for the two study groups. Nor was the slope of retention from the inner zone of the lung between 0 and 1 h and 2 and 6 h different for the two study groups.

Figure 5 shows the mean slopes of retention in the inner zone of the lung between 1 and 2 h at baseline and on Day 6 for the two study groups. By Day 6, the mucociliary clearance rate in the inner zone of the right lung during this period of time was significantly greater for the rhDNase-treated patients than for the placebo group. At baseline, the mean slope of retention was −5.51 ± 2.4 for the rhDNase-treated group. This meant that an average of 5.51% of the radiolabel was cleared from the lung

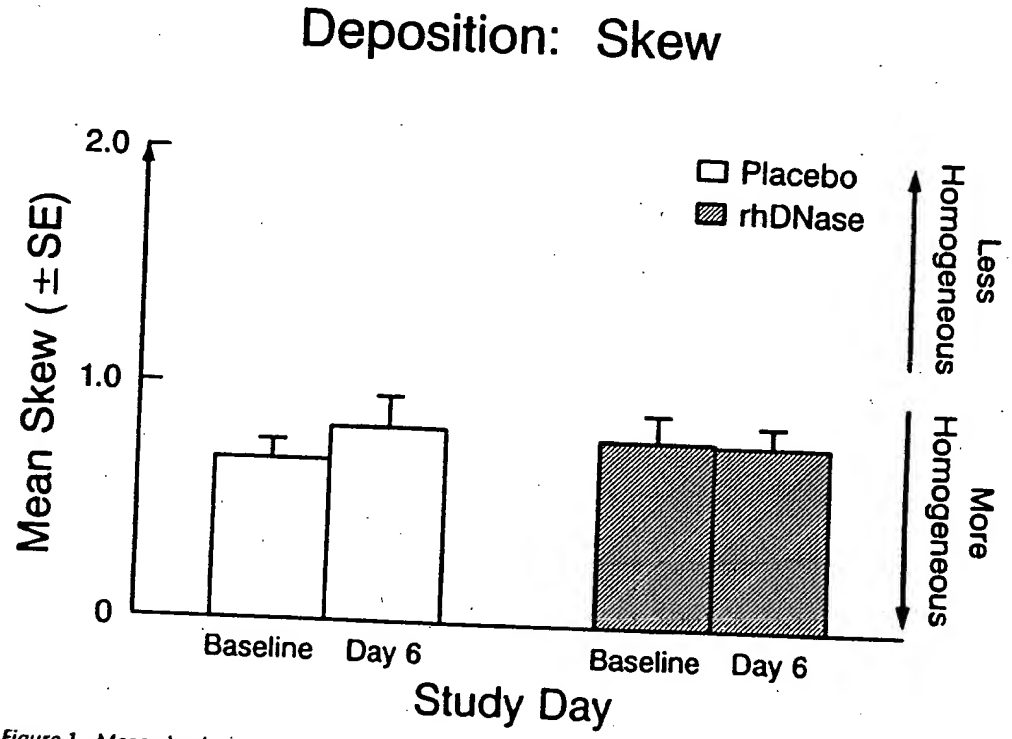

Figure 1. Mean absolute values for skew (± SE) for placebo- (*white bars*) and rhDNase-treated (*gray bars*) patients at baseline and Day 6. There were no statistically significant differences between the treatment groups for skew.

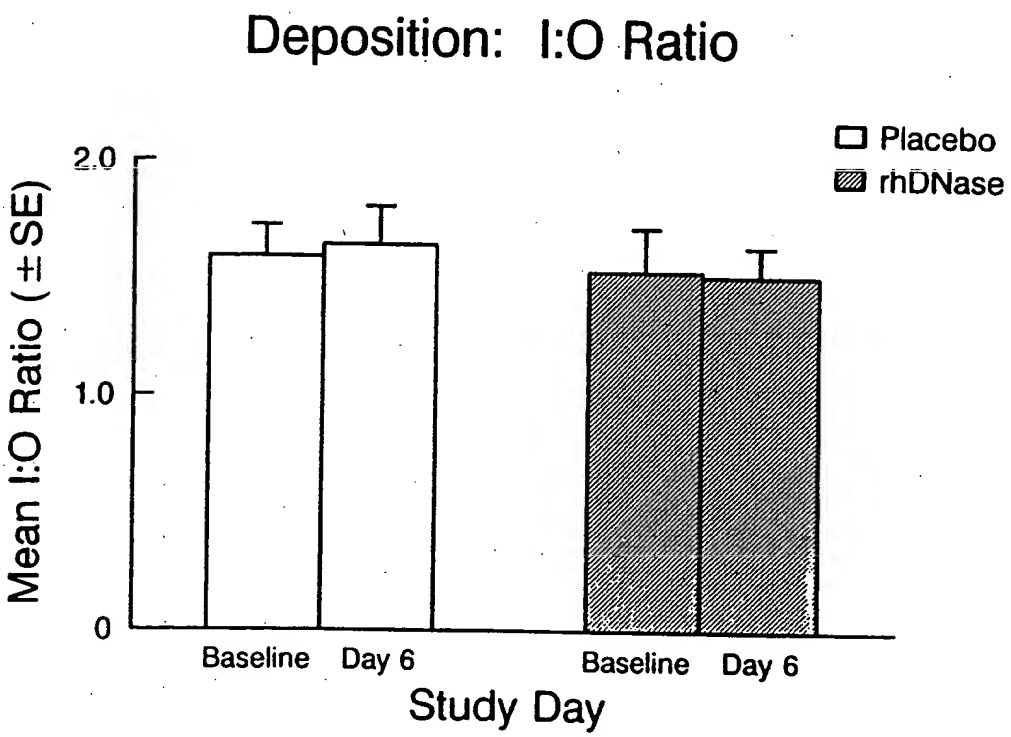

Figure 2. Mean absolute values for I:O ratio (± SE) for placebo- (*white bars*) and rhDNase-treated (*gray bars*) patients at baseline and Day 6. There were no statistically significant differences between the treatment groups for I:O ratio.

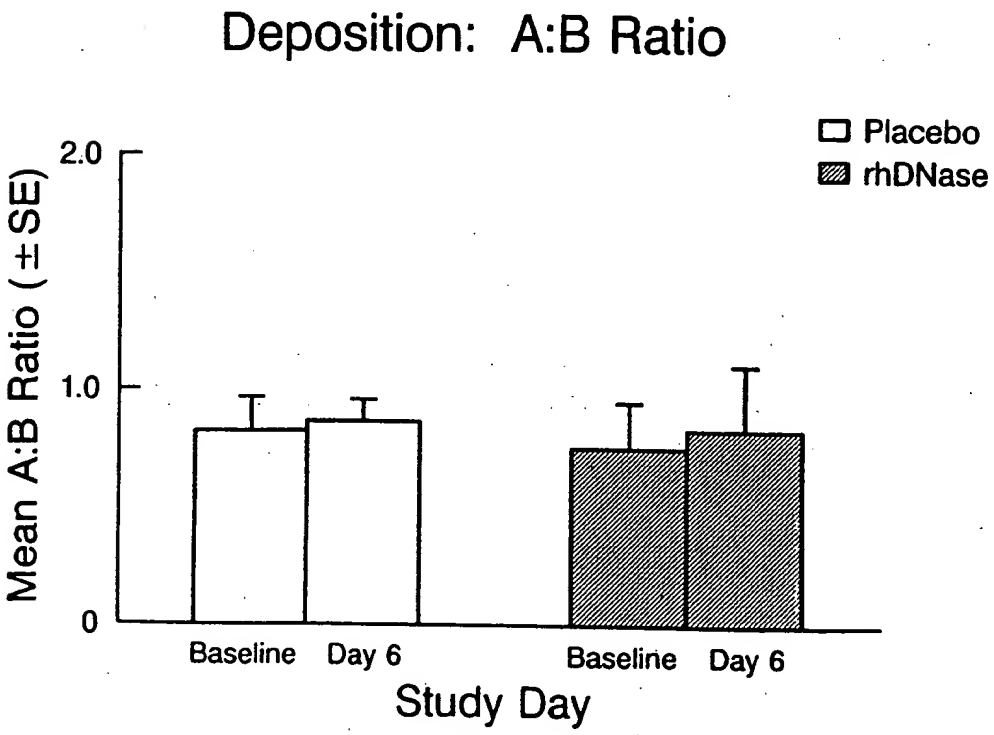

Figure 3. Mean absolute values for A:B ratio (± SE) for placebo- (*white bars*) and rhDNase-treated (*gray bars*) patients at baseline and Day 6. There were no statistically significant differences between the treatment groups for A:B ratio.

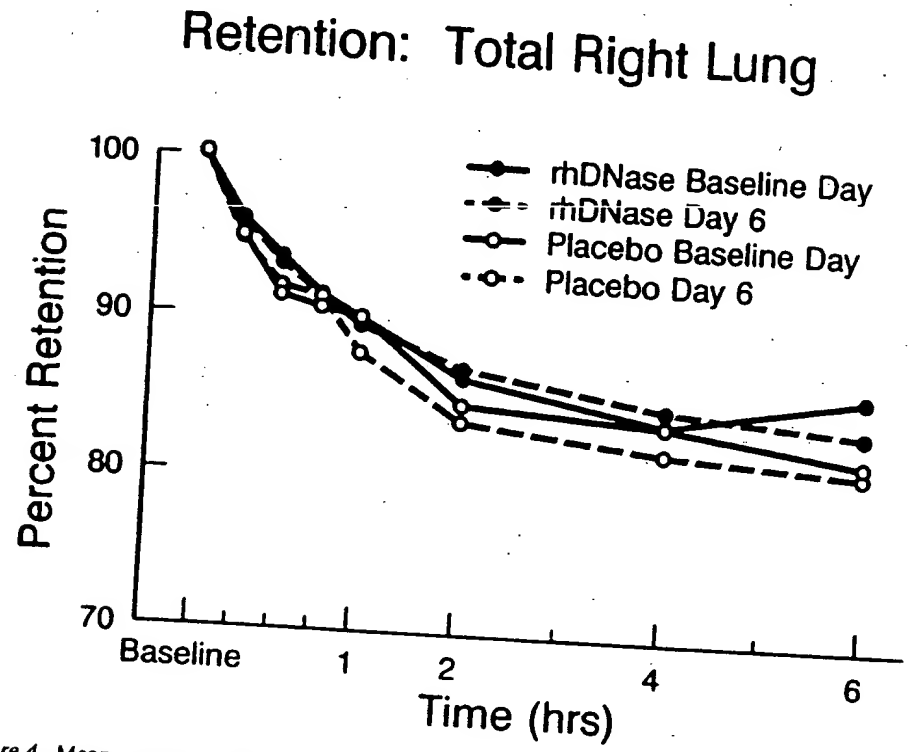

Figure 4. Mean percent retention of the radiolabel in the total right lung at 15, 30, 45, and 60 min and 2, 4, and 6 h after inhalation for placebo- (*open circles*) and rhDNase-treated (*closed circles*) patients at baseline and Day 6. There were no significant changes in retention from baseline to Day 6 between the treatment groups.

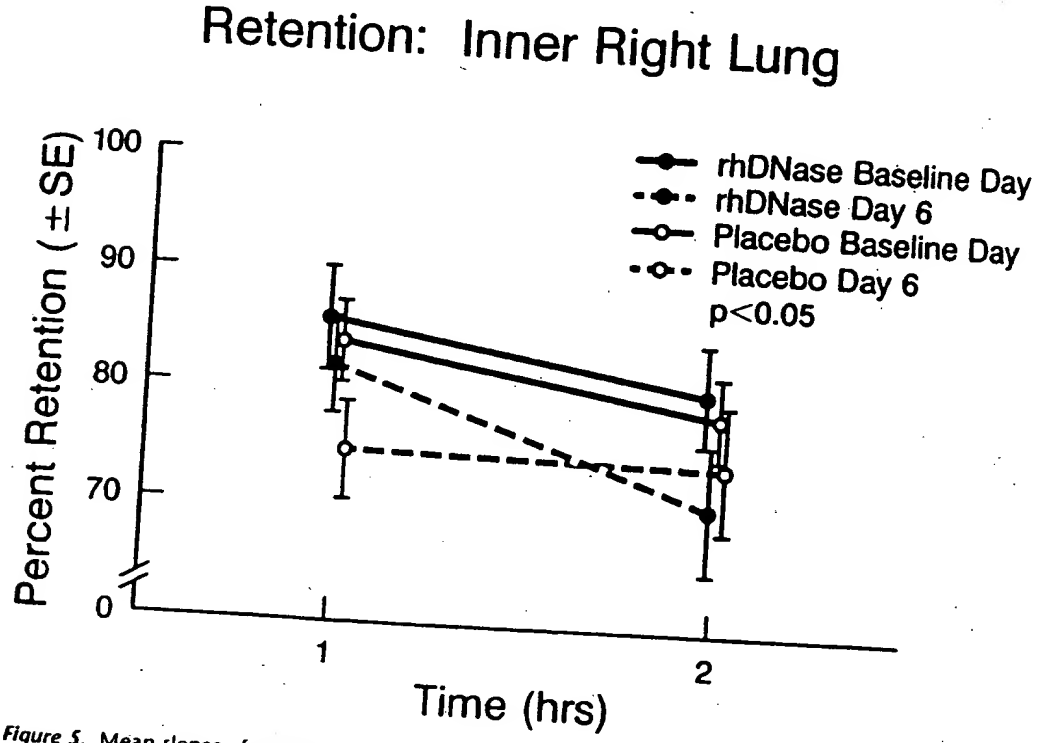

Figure 5. Mean slopes of retention of the radiolabel in the inner zone of the right lung between 1 and 2 h after inhalation for placebo- (*open circles*) and rhDNase-treated (*closed circles*) patients at baseline and Day 6. By Day 6, the slope of retention at this time period was significantly steeper in the rhDNase-treated group than in the placebo-treated group, indicating faster clearance.

over this time period. By Day 6, the mean slope of retention for the same time period was −11.49, a change of −5.98 ± 3.43. This slope indicated that an average of 11.49% of the radiolabel had cleared from the inner zone of the right lung in the rhDNase-treated group on Day 6. For the placebo group, the mean slope of retention was −5.31 ± 3.00 at baseline. By Day 6, the mean slope of retention was −0.57, a change of +4.74 ± 2.88, indicating than an average of only 0.57% of the radiolabel had cleared. A comparison of the change in retention from baseline to Day 6 for the two groups indicated that clearance from the inner zone was significantly enhanced in the rhDNase-treated group over this time period.

Changes in Cough Frequency

All patients coughed during the 6 h after administration of the radioaerosol. The average number of coughs over the 6-h period for the rhDNase-treated patients (15.3 ± 2.2) and the placebo patients (21.9 ± 5.5) at baseline was not significantly different. By Day 6, the number of coughs decreased by an average of 29% in the rhDNase-treated group and increased by an average of 6% in the placebo group. However, these differences were not statistically significant.

Pulmonary Function Changes

For the placebo group and rhDNase-treated patients, mean FEV_1 values at baseline were 58.8 ± 3.6% and 50.9 ± 6.2% predicted, respectively, which were not significantly different. By Day 6, FEV_1 increased by an average of 9.4 ± 3.5% in the rhDNase-treated patients and decreased by an average of 1.8 ± 1.7% in the placebo group ($p < 0.05$).

The FVC values averaged 76.9 ± 4.5% and 70.9 ± 5.5% predicted for the placebo and rhDNase-treated groups at baseline, which were not significantly different. By Day 6, the FVC increased by an average of 12.7 ± 2.6% in the rhDNase-treated patients and increased by an average of 0.4 ± 1.1% in the placebo group ($p < 0.05$).

Although FEV_1 and FVC both improved following rhDNase treatment, there was no significant change in the FEV_1/FVC ratio on Day 6 (0.68 ± 0.05) as compared with baseline (0.70 ± 0.05) in the rhDNase-treated patients.

CPT Effects

By Day 2, FEV_1 and FVC values in the placebo group decreased significantly after CPT compared with values for the rhDNase-treated group. The FEV_1 decreased by an average of 4.6%, whereas the FVC decreased by an average of 3.6%. In the rhDNase-treated group, the FEV_1 increased by an average of 5.1% ($n = 8$) and the FVC increased by an average of 4.1% ($n = 8$) for the same time period. By Day 6, however, there was a decrease in FEV_1 and FVC values, respectively, in both study groups after CPT. The mean percent changes were −4.7 ± 1.3 and −3.5 ± 1.4 for FEV_1 and FVC, respectively, in the placebo group, and −1.3 ± 2.0 and −0.2 ± 1.3, respectively, in the rhDNase-treated group. These decreases, however, were not significant.

DISCUSSION

Effect of rhDNase on Airflow Obstruction

Prior to the study, we hypothesized that rhDNase treatment alters CF mucus such that regional and/or local obstruction to airflow within the lungs, as measured by indices of aerosol deposition homogeneity, is reduced, and that this reduction is associated with improvement in pulmonary function. Results from this clinical trial do not support this hypothesis. Measurements of pulmonary function did show significant improvement by Day 6 in the rhDNase-treated patients compared with the placebo group. The FVC in the rhDNase-treated patients increased by an average of 12.7% and the FEV_1 increased by an average of 9.4%. These findings are similar to those previously reported (5). However, the observed improvements in lung function were not accompanied by alterations in any of the aerosol-deposition parameters that are known to be affected by changes in airflow obstruction (i.e., I:O ratio, A:B ratio, or skew). Each of these parameters showed slight changes in aerosol-distribution uniformity, suggesting a possible decrease in airflow obstruction regionally or locally, but these alterations were not significantly different from those observed in the patients given placebo. High interpatient variability in these deposition parameters may have contributed to the lack of statistical differences between the two study groups. For example, the coefficient of variation (CV) for baseline skew values in the placebo group was 42%. Nevertheless, FVC measurements were more affected by rhDNase treatment than were FEV_1 measurements, which resulted in no significant change in the FEV_1/FVC ratio on Day 6 (0.68 ± 0.05) as compared with baseline (0.70 ± 0.05). Lack of improvement in this index of airflow obstruction further suggests that rhDNase may be acting by a mechanism other than a reduction in airflow obstruction. These results are dissimilar to those of Ramsey and colleagues (5), who reported a 1.2% increase in the FEV_1/FVC ratio in 44 patients with CF after 10 d of treatment with 2.5 mg of rhDNase twice daily. One explanation for this difference may involve the larger patient population in the study by Ramsey and colleagues (5) or the longer treatment period in their study. Regardless of the explanation, neither the small percent increase in the FEV_1/FVC ration in the study of Ramsey and colleagues nor the small percent decrease in the ratio in our study support the hypothesis that the mechanism of action of rhDNase is to reduce airflow obstruction in CF patients. It is unknown whether rhDNase reduces airflow obstruction in airways too small to affect changes measured by FEV_1, FVC, or indices of aerosol-deposition homogeneity.

Results from the present study are in some ways similar to those obtained in our previous study of CF patients treated in the hospital for an average of 14 d for a pulmonary exacerbation of CF (13). Twelve of the 20 patients in our previous study had a mean baseline FEV_1 of 49% predicted, a mean FVC of 62% predicted, and a mean skew of 0.81. These values are similar to those observed in the 10 rhDNase-treated patients in the present study. In the 12 patients, FEV_1 improved by 12.2% and FVC improved by 11.3% following hospitalization. However, skew improved by only 0.01 units, and the FEV_1/FVC ratio improved by only 1.4%.

In contrast, eight patients in our previous study had a lower mean baseline FEV_1 (19% predicted) and FVC (31% predicted) and a higher mean skew (1.27) than the patients in the present study. Those patients not only demonstrated significant improvement in FEV_1 (26.3%) and FVC (38.7%), but also showed a mean decrease of 0.37 units in skew and a mean increase in the FEV_1/FVC ratio of 19.2%, suggesting a reduction in airflow obstruction as a result of their treatment during hospitalization.

It is unknown whether CF patients with baseline pulmonary function values similar to the eight patients in our previous study would demonstrate significant improvement in indices of aerosol-deposition uniformity and FEV_1/FVC ratio after rhDNase treatment. Results from the study by Ramsey and colleagues (5) suggest that this might be the case, since the greatest improvement in pulmonary function in that study was experienced by patients who had more severe pulmonary disease (FVC < 70% predicted). Alternatively, rhDNase may have its major effect in improving the health status of CF patients by increasing the FVC and thereby improving gas exchange, perhaps by reducing the residual volume caused by air trapping.

Effect of rhDNase on Mucociliary Clearance

Mucociliary clearance can be quantified by inhalation of a radiolabeled aerosol followed by sequential gamma imaging of the lungs. However, measurements of clearance can be confounded by the site of radioaerosol deposition within the lungs. Clearance from the proximal airways is faster than clearance from distal airways because of the differences in the length of the mucociliary pathway. Abnormalities that limit airflow through the airways, such as an increase in the thickness of the mucus layer or mucoid impaction, can result in more proximal deposition of the radioaerosol and the measurement of faster clearance rates. Relief of airflow limitation can result in a more distal distribution of deposited aerosol and a decrease in the measured clearance rate. Theoretically, an agent that reduces airway mucus viscosity could reduce airflow limitation and improve the deposition of an inhaled aerosol in the distal airways, thereby leading to a slowing in the mucociliary clearance rate. For this reason, we utilized aerosol deposition parameters (i.e., I:O ratio, A:B ratio, and skew) for a second purpose: to quantify reproducibility in the deposition pattern prior to determining the effects of rhDNase on mucociliary clearance.

We assumed two possible effects of rhDNase treatment on deposition reproducibility in our experimental design. First, we hypothesized that airflow obstruction would be reduced and that deposition indices would improve as a result of treatment. For reasons stated earlier, this outcome would necessarily invalidate any concomitant measurement of mucociliary clearance. On the other hand, if deposition indices did not change as a result of treatment, measurement of changes in mucociliary clearance would be valid.

The effect of rhDNase on mucociliary clearance is less certain than its effect on airflow obstruction. After quantifying mucociliary clearance over a 6-h period at baseline, we found no significant improvement in clearance after 6 d of rhDNase treatment. This lack of improvement could not be attributed to more distal deposition of the radioaerosol on Day 6 versus baseline, because deposition indices did not change. The mean percent retentions of the radioactive marker after 6 h at baseline and after 6 d of placebo treatment were 82% and 81%, respectively. These values were not significantly different from those for the rhDNase-treated patients, who demonstrated 86% and 84% retention at the same time points at baseline and Day 6, respectively. At baseline and Day 6, percent retention after 1 h averaged approximately 90% in both the placebo group and the rhDNase treated-patients. These findings are consistent with those of Regnis and associates (21) and Mortensen and coworkers (22). They demonstrated that the mean percent retention after 1 h in control patients with CF, who had baseline characteristics similar to those in our study, was approximately 90%.

One confounding variable in the interpretation of the clearance data in our study was the difference in the amount of coughing for the placebo and rhDNase-treated groups. All patients coughed during the 6 h after administration of the radioaerosol. However, by Day 6, the number of coughs decreased in the rhDNase-treated group and increased slightly in the placebo group. In the study by Ramsey and colleagues (5), patients also perceived a decrease in cough frequency and severity while being treated with rhDNase. By continuing to cough at the same or slightly greater frequency, the placebo group in our study may have cleared the radioactive marker mechanically rather than by mucociliary clearance mechanisms. This method of removal could have resulted in similar percent clearance measurements when compared with the rhDNase-treated patients, yet the mechanisms of removal would be different. Future studies in which the degree of coughing is matched for patients given placebo and for rhDNase-treated patients might eliminate this variable, thereby providing a more accurate measurement of the effect of rhDNase on mucociliary clearance. In addition, we quantified mucociliary clearance after only 6 d of treatment with rhDNase. Perhaps changes in mucociliary clearance would be detectable after a long treatment period (i.e., several weeks).

Although the rate of mucociliary clearance from the inner zone of the lung, as measured by the slope of retention during the 1- to 2-h period after aerosol inhalation, was significantly increased on Day 6 in the rhDNase-treated group, this rate of increase did not continue over the next 4 h of measurement. These findings may indicate that a primary effect of rhDNase is to improve mucociliary clearance in the first few hours after treatment. Future studies with larger numbers of subjects are needed to validate this observation.

When measuring mucociliary clearance with radioaerosols, some investigators (20) prefer to target deposition in the large, central airways, because these airways are highly ciliated and clearance time is the most rapid. We believe that the most important determinant of the site of deposition of a radioaerosol chosen to measure changes in mucociliary clearance is the site of action of the agent under study. When taking rhDNase aerosol, patients were instructed to inhale slowly, beginning from functional residual capacity (FRC). For this reason, we delivered the radioaerosol with the same nebulizer, instructing the patients to inhale slowly (30 L/min), starting from 50% of their vital capacity (VC). In this way, we targeted deposition of the radioaerosol to the area in which the rhDNase was most likely to deposit.

There is no reason to believe that the radioaerosol and rhDNase aerosol were depositing differently within the lungs, since the breathing maneuvers were similar and the particle-size characteristics of the two aerosols were similar. The median particle sizes for the rhDNase aerosol and the radioaerosol were 4.47 and 3.78 μm, respectively. The ~ 0.7 μm difference in particle size was due to the use of the dosimeter in line with the compressor when radioaerosol was generated. It is unlikely that this small difference produced significantly different deposition patterns.

Effect of CPT on Changes in FEV_1 and FVC

By Day 6, the FEV_1 increased by an average of 9.4% and the FVC increased by an average of 12.7% in the rhDNase-treated patients. These values were significantly higher than those for the patients who received placebo, who demonstrated a mean decrease in FEV_1 of 1.8% and a mean increase in FVC of 1.1%. Both treatment groups showed decreases in FEV_1 and FVC after CPT, but the changes were not significant. Results from this study therefore indicate that the significant improvements in FEV_1 and FVC observed in the rhDNase-treated patients versus those given placebo were unrelated to CPT.

In summary, the results of the present study indicate that improvements seen in FEV_1 and FVC after rhDNase treatment are independent of CPT. We were unable to demonstrate that rhDNase reduces airflow obstruction, as quantified by changes in spirometric measurements and aerosol deposition homogeneity, or improves mucociliary clearance.

References

1. Collins, F. S., J. R. Riordan, and L. C. Tsui. 1990. The cystic fibrosis gene: isolation and significance. *Hosp. Pract.* 25:47–57.
2. White, J. C., and P. C. Elmes. 1958. The rheological problem in chronic bronchitis. *Reologic Acta* 1:96–102.
3. Potter, S. L., S. Spector, L. W. Matthews, and J. Lemm. 1969. The nucleic acids in whole pulmonary secretions from patients with cystic fibrosis, bronchiectasis and laryngectomy. *Am. Rev. Respir. Dis.* 99: 909–916.
4. Shak, S., D. J. Capon, R. Hellmiss, S. A. Marstas, and C. L. Baker.

1990. Recombinant human DNase reduces the viscosity of cystic fibrosis sputum. *Proc. Natl. Acad. Sci. U.S.A.* 87:9188–9192.
5. Ramsey, B. W., S. J. Astley, M. L. Aitken, W. Burke, A. A. Colin, H. L. Dorkin, J. D. Eisenberg, R. L. Gibson, I. R. Harwood, D. V. Schidlow, R. W. Wilmott, M. E. Wohl, L. J. Meyerson, S. Shak, H. Fuchs, and A. L. Smith. 1993. Efficacy and safety of short-term administration of aerosolized recombinant human deoxyribonuclease in patients with cystic fibrosis. *Am. Rev. Respir. Dis.* 148:145–151.
6. Fuchs, H. J., D. S. Borowitz, D. H. Christiansen, E. D. Morris, M. I. Nash, B. W. Ramsey, B. J. Rosenstein, A. L. Smith, and M. E. Wohl. 1994. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. *N. Engl. J. Med.* 331:637–642.
7. Laube, B. L., D. L. Swift, H. N. Wagner, Jr., P. S. Norman, and G. K. Adams, III. 1986. The effect of bronchial obstruction on central airway deposition of a saline aerosol in patients with asthma. *Am. Rev. Respir. Dis.* 133:740–743.
8. Dolovich, M. B., J. Sanchis, C. Rossman, and M. T. Newhouse. 1976. Aerosol penetrance: a sensitive index of peripheral airways obstruction. *J. Appl. Physiol.* 40:468–471.
9. Chung, K. F., K. Jeyasingh, and P. D. Snashall. 1988. Influence of airway calibre on the intrapulmonary dose and distribution of inhaled aerosol in normal and asthmatic subjects. *Eur. Respir. J.* 1:890–895.
10. Agnew, J. E., J. R. M. Bateman, D. Pavia, and S. W. Clarke. 1984. Radionuclide demonstration of ventilatory abnormalities in mild asthma. *Clin. Sci.* 66:525–531.
11. Laube, B. L., J. M. Links, H. N. Wagner, Jr., P. S. Norman, D. W. Koller, N. D. LaFrance, and G. K. Adams, III. 1988. Simplified assessment of fine aerosol distribution in human airways. *J. Nucl. Med.* 29:1057–1065.
12. Laube, B. L., J. M. Links, N. D. LaFrance, H. N. Wagner, Jr., and B. J. Rosenstein. 1989. Homogeneity of bronchopulmonary distribution of ^{99m}Tc aerosol in normal subjects and in cystic fibrosis patients. *Chest* 95:822–830.
13. Laube, B. L., D. Y. Chang, A. N. Blask, and B. J. Rosenstein. 1992. Radioaerosol assessment of lung improvement in cystic fibrosis patients treated for acute pulmonary exacerbations. *Chest* 101:1302–1308.
14. Brasfield, D., G. Hicks, S.-J. Soong, and R. E. Tiller. 1979. The chest roentgenogram in cystic fibrosis: a new scoring system. *Pediatrics* 63:24–29.
15. Laube, B. L., P. S. Norman, and G. K. Adams, III. 1992. The effect of aerosol distribution on airway responsiveness to inhaled methacholine in patients with asthma. *J. Allergy Clin. Immunol.* 89:510–518.
16. Laube, B. L., S. M. Bowes, III, J. M. Links, K. K. Thomas, and R. Frank. 1993. Acute exposure to acid fog: effects on mucociliary clearance. *Am. Rev. Respir. Dis.* 147:1105–1111.
17. American Thoracic Society Standardization of Spirometry—1987 Update. 1987. *Am. Rev. Respir. Dis.* 136:1285–1298.
18. Knudson, R. J., M. D. Lebowitz, C. J. Holberg, and B. Burrows. 1983. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am. Rev. Respir. Dis.* 127:725–734.
19. Snedecor, G. W., and W. G. Cochran. Statistical Methods. 1967. Iowa State University Press, Ames, IA. 100–300.
20. Taussig, L. M., J. Katwinkel, W. T. Friedewald, and P. A. di Sant'Agnese. 1973. A new prognostic score and clinical evaluation system for cystic fibrosis. *J. Pediatr.* 82:380–390.
21. Regnis, J. A., M. Robinson, D. L. Bailey, P. Cook, P. Hooper, H.-K. Chan, I. Gonda, G. Bautovich, and P. T. P. Bye. 1994. Mucociliary clearance in patients with cystic fibrosis and in normal subjects. *Am. J. Respir. Crit. Care Med.* 150:66–71.
22. Mortensen, J., M. Falk, S. Groth, and C. Jensen. 1991. The effects of postural drainage and positive expiratory pressure physiotherapy on tracheobronchial clearance in cystic fibrosis. *Chest* 100:1350–1357.

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.